--84. (New) A substantially pure subunit of the human neuronal nicotinic acetylcholine receptor encoded by the molecule of claim 81.--

REMARKS

In accordance with the present invention, there are provided isolated nucleic acids encoding human neuronal nicotinic acetylcholine receptor subunits, nucleic acids capable of hybridizing thereto, nucleic acid probes derived therefrom, isolated mRNA complementary thereto, transformed cells capable of expressing the protein products encoded by invention nucleic acids, as well as the proteins encoded by nucleic acids of the present invention.

By the present Communication, 58 and 68 have been amended and new claims 76-84 have been added to define Applicants' invention with greater particularity. The amendments to claims 58 and 68 were made to obviate the Examiner's assertion that these claims allegedly recite improper Markush groups. No new matter is introduced by the amendments, as the amended claim language is fully supported by the specification and original claims.

The aforementioned amendments are submitted to place the claims in condition for allowance, or alternatively, to present the claims in better condition for appeal. Such amendments were necessitated to clarify the scope of the invention in response to issues raised in the April 25, Final Office Action. The amendments submitted herewith were not made earlier because further clarification of the claim language was

not believed to be necessary to overcome the grounds of rejection initially raised in the July 27, 1994 Office Action. Therefore, entry of the amendments to claims 58 and 68 and of new claims 76-84 is respectfully requested.

Claims 53-63, 66-68 and 70-84 are currently under examination.

I. REJECTIONS AND OBJECTION UNDER 35 U.S.C. §112

Claims 53-63, 66-68 and 70-75 stand rejected and the specification stands objected to under 35 U.S.C. §112, first paragraph because the specification is allegedly only enabling for those DNAs contained within the clones identified by the ATCC accession numbers presented on page 19 of the specification. This rejection/objection is respectfully traversed.

Applicants respectfully disagree with the Examiner's assertion, set forth on page 3, lines 5-10 of Paper No. 15, that in the absence of an explicit teaching in Applicants' specification of which particular amino acid residues are essential and which residues are either expendable or substitutable, one of ordinary skill in the art would have to

> resort to a substantial amount of undue experimentation in the form of insertional, deletional and substitutional mutation analysis of over 400 amino acid residues before they could even begin to rationally design a functional human neuronal nicotinic acetylcholine receptor subunit having other than a natural amino acid sequence.

Functional neuronal nicotinic acetylcholine receptors are known to arise from the co-expression of multiple subunits (e.g., α- and ß-subunits).[1] It is also known that α-subunits serve as agonist-binding subunits based on their structural homology to the muscle α-subunit and ß-subunits serve as structural units.[2] Applicants provide details defining the structure of these subunits (see, for example, page 19, line 1 through page 20, line 21; page 21, lines 7-11; page 22, lines 15-25; page 23, lines 21-26; and Figures 1-9).

Indeed, a review of the art at the time of the present invention reveals that skilled artisans were capable of identifying the amino acid residues (and the nucleic acids encoding same) of a protein that could be modified without rendering the encoded protein (i.e., HnAChRα2, HnAChRα3 and HnAChRß2) non-functional.[3] More specifically, however, these

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[1]. See, for example, Connolly, J.G., "Mini review: Structure-function Relationships in Nicotinic Acetylcholine Receptors" *Comparative Biochemistry and Physiology* A93:221-231 (1989); and Couturier et al., *Neuron* 15(6):847-856 (1990).

[2]. See, for example, Boulter et al., *Nature* 319:368-374 (1986); Deneris et al. *Neuron* 1:45-54 (1988); and Duvoisin et al., *Neuron* 3:487-496 (1989).

[3]. See, for example, Bowie and Sauer, "Identifying Determinants of Folding and Activity for a Protein of Unknown Structure" *Proc. Natl. Acad. Sci., USA* 86:2152-2156 (1989); Taylor, W.R., "Identification of Protein Sequence Homology by Consensus Template Alignment" *J. Mol. Biol.* 188:233-258 (1986); Buetti and Kühnel, "Distinct Sequence Elements Involved in the Glucocorticoid Regulation of the Mouse Mammary Tumor Virus Promoter Identified by Linker Scanning Mutagenesis" *J. Mol. Biol.* 190:379-389 (1986); Sprang et al., "The Three-Dimensional Structure of Asn^{102} Mutant of Trypsin: Role of Asp^{102} in Serine Protease Catalysis" *Science* 237:905-909 (1987); and Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions" *Science*

same artisans were, in fact, capable of producing such nucleic acids without undue experimentation.[4]

The specification stands further objected to under 35 U.S.C. § 112, first paragraph, as allegedly failing to provide an enabling disclosure for the production of a substantially pure subunit of a human neuronal nicotinic acetylcholine receptor. This objection is respectfully traversed.

Applicants respectfully disagree with the Examiner's assertion that transmembrane proteins

> would be as difficult to recover from a heterologous host as they would from the cells in which they are naturally produced.

See page 5, lines 21-23 of Paper No. 15. It is respectfully submitted that invention subunits can readily be produced using various methods well known to skilled artisans. An example of a commonly employed means to produce individual subunit(s) and/or functional receptors is to express nucleic acids encoding such subunits and/or receptors in a suitable host cell. Applicants' specification describes various host cell systems in a manner such that those of skill in the art can readily identify host cells suitable for use in the practice of the present invention.

247:1306-1310 (1990).

[4]. See, for example, Cooper et al., "Pentameric Structure and Subunit Stoichiometry of a Neuronal Nicotinic Acetylcholine Receptor" *Nature* 350:235-238 (1991); Lo et al., "Role of a Key Cysteine in the Gating of the Acetylcholine Receptors" *Neuron* 5:857-866 (1990); and Revah et al., "Mutations in the Channel Domain Alter Desensitization of a Neuronal Nicotinic Receptor" *Nature* 353:846-849 (1991).

Indeed, host cells suitable for use in the practice of the present invention are well-known and widely used by molecular and cellular biologists. Moreover, such host cell systems are commercially available. Accordingly, Applicants need not burden the specification with that which is well-known in the art.

Notwithstanding, responsive to the Examiner's request for references describing

> the recovery of a structurally related protein from a heterologous host prior to the filing of the instant application. . .

See page 5, line 23 through page 6, line 3 of Paper No. 15. Applicants attach hereto, as Exhibit A, copies of two references that describe the recovery of nicotinic acetylcholine receptor subunits from heterologous host cells: Claudio et al., "Genetic Reconstitution of Functional Acetylcholine Receptor Channels in Mouse Fibroblasts" *Science* 238:688-694 (1987) and Whiting et al., "Structural and Pharmacological Characterization of the Major Brain Nicotinic Acetylcholine Receptor Subtype Stably Expressed in Mouse Fibroblasts" *Mol. Pharm.* 40:463-472 (1991).

A specification is enabled under 35 U.S.C. § 112 if it provides sufficient information that permits one skilled in the art to make and use the claimed invention without the exercise of undue experimentation. The specification is directed to those skilled in the art to which the invention pertains, and thus need not laboriously detail that which is common and well known in the art. Spectra-Physics, Inc. v. Coherent, Inc., 3 USPQ2d 1737 (Fed. Cir. 1987).

In all instances described hereinabove, Applicants provide substantial enablement to the skilled artisan for the practice of the present invention. Applicants respectfully submit, therefore, that the claims under examination are, indeed, enabled in the specification such as to allow one of ordinary skill in the art, using well-known techniques, to make **and** use the claimed invention without undue experimentation.

In view of the above remarks, reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, is respectfully requested.

II. REJECTIONS UNDER 35 U.S.C. § 103

Claims 53, 54, 57-63, 66-68, 70 and 72-75 stand rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Boulter et al., *Proc. Natl Acad. Sci., USA* 84:7763-7767 (1987) in view of Grenningloh et al., *EMBO J.* 9(3):771-776 (1990), Schofield et al., *FEBS Letters* 244(2):361-364 (1989), and Noda et al., *Nature* 305:818-823 (1983). This rejection is respectfully traversed.

Applicants' invention, as defined by all of the claims, distinguishes over the art by requiring isolated DNA encoding **human** neuronal nACh receptor subunits. Specifically, Applicants' invention, as defined by claims 53, 54, 57, 58, 73-75 and 79-81, distinguishes over the art by requiring an isolated (or substantially pure) nucleic acid molecule encoding a **human** neuronal nAChR subunit (alpha2, alpha3, or beta2). Applicants' invention, as defined by claims 65-70, further distinguishes over the art by providing transformed host cells that express

invention receptor subunit DNAs and RNAs, as well as methods for identifying compounds which bind to invention receptors. Additionally, Applicants' invention, as defined by claim 72, further distinguishes over the art by providing a method for making cells having **human** neuronal nicotinic acetylcholine receptor activity. Applicants' invention, as defined by claim 71, further distinguishes over the art by providing substantially pure **human** neuronal nACh receptors comprising at least one **human** alpha and one **human** beta subunit.

Boulter et al. do not teach or suggest a **human** neuronal nACh receptor or subunit thereof. Furthermore, Boulter et al. neither teaches nor suggests the **rat** neuronal nAChR alpha2 subunit. Rather, Boulter et al. discloses cDNA clones encoding **rat** neuronal nACh receptor subunits alpha3, alpha4, and beta2. Boulter et al. do not teach or suggest transformed host cells that express **human** neuronal nACh receptors. Furthermore, Boulter et al. do not teach or suggest methods for identifying compounds which bind to **human** neuronal nACh receptors.

Applicants' invention is directed to DNA encoding **human** neuronal nAChR subunits. Only Applicants define specific **human** neuronal nAChR subunits. There was **no** disclosure in the art as to the nature or degree of differences between nucleic acid sequences encoding **human** neuronal nAChR subunits relative to subunits of other species. Only Applicants, having isolated and characterized **human** specific neuronal nAChR subunits, can teach the differences between nucleic acid sequences encoding **human** neuronal nAChR subunits from neuronal nAChR subunits of other species.

Boulter et al. neither teach nor suggest screening **human cDNA libraries** with a **rat** subunit clone. Accordingly, no guidance is provided with respect to the particular human tissue type library to be screened. Furthermore, Boulter et al. neither teach nor suggest which **rat clone** (or portion thereof) would be expected to be conserved (with respect to human sequence). Accordingly, Boulter neither teach nor suggest which rat clone (or portion thereof) would be potentially useful for screening other mammalian libraries. Boulter et al. does not teach or suggest hybridization conditions under which a rat cDNA probe could be used to screen a human library, nor what particular tissue type library to screen. The identification of suitable libraries for screening is especially significant because, as is well known in the art, the tissue distribution of neuronal receptors can be disparate between mammalian species.[5]

None of the secondary references, i.e., Grenningloh et al., Schofield et al., and Noda et al. are capable of curing the deficiencies of Boulter et al. Furthermore, the asserted combination of references, taken alone or in combination, does not teach or suggest invention receptors nor subunits thereof. Moreover, the evolutionary conservation and inter-species homology observed for individual glycine, $GABA_A$, and nicotinic acetylcholine receptor genes is neither suggestive nor

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[5]. See, for example, Larsson et al., J. Neural Transm. 69:3-18 (1987) (in contrast to rat brain there are multiple nicotinic acetylcholine receptors in human cerebral cortex); Moner et al., Science 256:1217-1221 (1992) (rat NMDAR2C is abundant in rat cerebellum, but is **not** abundant in rat hippocampus) (Compare with human NMDAR2C which **is** abundant in human hippocampus); and Abe et al., J. Biol. Chem. 267:13361-13368 (1992) (rat mGluR5 is abundant in rat hippocampus but is **not** abundant in rat cerebellum, whereas human mGluR5 **is** abundant in human cerebellum).

dispositive of inter-family and/or inter-species characteristics of invention receptors. Clearly, determination of species differences can be made only **after** the human clone has been characterized, with the improper benefit of hindsight. See, W.L. Gore & Associates, Inc. v. Garlock, Inc., 220 USPQ 303, 313 (Fed. Cir. 1983).

Grenningloh et al., for example, do not teach or suggest a human **neuronal nACh** receptor or subunit thereof. Instead, Grenningloh et al. disclose human **glycine** receptor subunits. Contrary to the Examiner's assertion (page 8, line 24 through page 9, line 1 of Paper No. 15), Grenningloh et al. do **not** teach the members of the ligand-gated ion channel receptor superfamily are **functionally** related. Instead, Grenningloh et al. disclose that the receptors "exhibit a distinct pharmacology" (page 771, paragraph 1).

Further, Applicants respectfully disagree with the Examiner's assertion that

> no reference has been cited or made of record which reported less than 95% amino acid sequence identity between homologous receptor subunits from different mammalian species prior to the making of the instant invention. . .

See page 9, lines 12-15 of Paper No. 15. The Examiner's attention is directed to the disclosure in Grenningloh et al. of a **human** glycine receptor α subunit having only **76%** amino acid sequence identity to the homologous **rat** glycine receptor α subunit (page 771, Abstract and Results). Accordingly, the Examiner is respectfully submitted to be in error in dismissing Applicants' assertion that there are inter-species subunits that

are **not** suitable paradigms for purposes of analogy or comparison. Thus, rat/human glycine receptor subunits are clearly seen to be unsuitable paradigms of analogy or comparison.

Schofield et al. do not teach or suggest a human **neuronal nACh** receptor or subunit thereof. Instead, Schofield et al. disclose human $GABA_A$ receptor subunits. Schofield et al. neither teach nor suggest screening **human cDNA libraries** with a **rat** subunit clone. Schofield et al. do not teach or suggest hybridization conditions under which a rat cDNA probe could be used to screen a human cDNA library, nor what particular tissue type library to screen.

Noda et al. do not teach or suggest a human **neuronal** acetylcholine receptor. Instead, Noda et al. disclose a human **muscle** acetylcholine alpha subunit precursor. Noda et al. neither teach nor suggest screening **human cDNA libraries** with a **rat** subunit clone. Noda et al. do not teach or suggest hybridization conditions under which a rat cDNA probe could be used to screen a human cDNA library, nor what particular tissue type library to screen.

None of the above references disclose or suggest DNA encoding any subunits of **human** neuronal nAChRs. None of the cited references disclose or suggest anything regarding the relationship of the particular rat subunit to any human subunits. None of the references relied upon, taken alone or in combination, provide enabling methodology for identifying the claimed DNAs, nor for identifying particular libraries to screen to obtain a particular receptor type subunit, nor for the isolation of DNA encoding a human neuronal nAChR subunit.

In concluding that the claimed DNAs are obvious, the Examiner cannot look to the instant specification; to do so involves improper use of hindsight. The Examiner must only rely on the disclosures in the prior art. Until such DNAs were isolated, their sequences were unknown. It is respectfully submitted to be clear, therefore, that the Examiner has improperly chosen portions of the aforementioned references with benefit of hindsight in order to support the allegation of obviousness.

It is well established that a prior art reference must be taken in its entirety, **including** those portions which argue against obviousness. Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc., 230 USPQ 416, 420 (Fed. Cir. 1986). Indeed, it is impermissible within the framework of 35 U.S.C. § 103 to pick and choose from a reference only so much of it as will support a conclusion of obviousness to the exclusion of other parts necessary to a full appreciation of what the reference fairly suggests to one skilled in the art. *Id.* at 419. See, also, Akzo N.V. v. International Trade Commission, 1 USPQ2d 1241, 1246 (Fed. Cir. 1986) (prior art references must be read as a whole and consideration must be given "where the references diverge and teach away from the claimed invention."). Accordingly, the combination of references cited by the Examiner does not disclose or suggest the instantly claimed invention.

Moreover, the Examiner's assertion (page 7, line 24 through page 8, line 6 of Paper No. 15) that

> DNA encoding a human acetylcholine receptor subunit . . . does not differ from those DNAs encoding the rat acetylcholine receptor subunits of Boulter et al. . . . in any

> unexpected manner as shown by the sequence comparisons presented in . . . the instant specification. . . [and] further supported by the data presented in Table I of the declaration by Edwin C. Johnson under 37 C.F.R. §1.132 . . .

is respectfully submitted to be improperly based upon hindsight analysis. Whether rat and human neuronal nicotinic acetylcholine receptors have "virtually identical" or distinct relative binding affinities for "three of the four ligands tested" can be determined only **after** the human clones have been isolated and the respective proteins have been functionally expressed. The results presented in Table I clearly demonstrate the inability to predict the functional properties of a human receptor on the basis of the properties of a receptor from another animal species. In addition, these results underscore the necessity of using **human** receptors in screening compounds for identification of potential new drugs to be **used in humans**.

Responsive to the Examiner's assertion that the receptors only differ in their affinities for nicotine (page 8, line 10 of Paper No. 15), the Examiner's attention is directed to page 7 of the declaration where it is indicated that Table I shows the relative magnitudes of the current responses to acetylcholine, nicotine, cytisine and DMPP **normalized** to the response to **1 μM acetylcholine** for each cell. The results of the same assays, with responses instead normalized to the response to 1 μM nicotine (see table below), clearly demonstrate that, contrary to the Examiner's assertion, the receptors also differ in their affinities for acetylcholine.

Relative Agonist Sensitivities: Rat vs. Human neuronal nAChR

	alpha2 beta2	
	human	rat
Nicotine	1.00	1.00
ACh	2.74	1.07

Skilled artisans recognize that differences in affinity for any ligand is dependent upon the primary amino acid sequence of the respective receptor. Therefore, amino acid sequence differences between rat and human receptors are seen to clearly effect differences in affinity for, and sensitivity to various ligands. Such differences can be determined only **after** the human receptors have been isolated.

Responsive to the Examiner's assertion (page 8, lines 11-13 of Paper No. 15) that

> [s]ince nicotine is not a naturally encountered neurotransmitter an artisan would not have expected receptor affinity for this ligand to be necessarily conserved between species. . .

it is respectfully submitted that the Examiner has not presented any evidence **why** one of ordinary skill in the art would expect similarities or differences in inter-species receptor affinity for naturally or "non-naturally" encountered neurotransmitters. In the absence of such evidence, it is respectfully submitted that the results of the assays presented (hereinabove and in the declaration) must be taken as unexpected. Alternatively, Applicants respectfully request that the Examiner provide support

for the above assertion in the form of an affidavit pursuant to 37 C.F.R. §1.107.

In view of the above remarks, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103, is respectfully requested.

III. SUMMARY

In view of the above amendments and remarks, reconsideration and favorable action on all pending claims is respectfully requested. If any questions or issues remain, the Examiner is invited to contact the undersigned at the telephone number set forth below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

10/25/95
Date

Stephen E. Reiter
Registration No. 31,192
Telephone: (619) 546-1995
Facsimile: (619) 546-9392

PRETTY, SCHROEDER,
BRUEGGEMANN & CLARK
444 South Flower Street, Suite 2000
Los Angeles, California 90071

Attachment: Exhibit A